IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

ATTY.'S DOCKET: KITAMURA=1

In re application of:	)	Art Unit: 1651
Hidetomo KITAMURA	)	Examiner:
Appln. No. 09/380,372	)	Washington, D.C.
Filed: September 1, 1999	)	
For: NOVEL CELL LINES AND SCREENING METHODS...	)	

DECLARATION UNDER 37 CFR 1.132

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

I, Hidetomo KITAMURA, a Japanese citizen, residing at 135, Komakado, 1-chome, Gotenba-shi, Shizuoka, 412-8513, Japan, hereby declare that I am the inventor of the above-entitled patent application, and that I received a D.V.M degree from Hokkaido University Faculty of Veterinary Medicine in March 1991.

I declare also that I have been employed by Chugai

Seiyaku Kabushiki Kaisha, the assignee of this application, and have been engaged in pharmaceutical research since May 1991 and that I work as a researcher for Pharmaceutical Research Laboratory II of Chugai Seiyaku Kabushiki Kaisha.

I also declare that I have read all of the Official Actions pertaining to the above-entitled application, and am familiar with each of the references cited in the Official Actions by the Examiner.

I declare further that the following experiment was conducted under my supervision and that the result is true and correct to the best of my knowledge.

Experiment

1. Methods

Effect of PTH on [^{3}H]thymidine uptake of CL-1 cells of the present invention was evaluated in the following method:

CL-1 cell line was derived from tibia of normal adult mouse as is disclosed in the specification of the present invention.

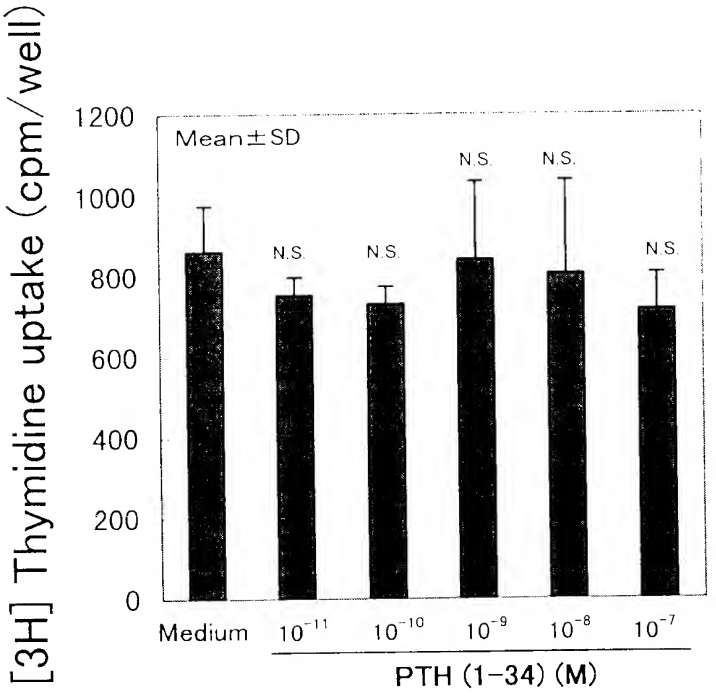
The CL-1 cells were grown to confluence in 96 well plastic plate (500 cells/well) with 0.2 mL α-MEM containing

100 μg/mL streptomycin, 100 U/mL penicillin and 3% fetal bovine serum under 5% CO_2 in air at 37°C. The CL-1 cells were then treated with 10^{-11} to 10^{-7} M PTH(1-34) in α-MEM containing 3% fetal bovine serum for 24 hours. After this treatment, a [^{3}H] thymidine solution (10 μL/well, 10 μCi/mL in PBS) was added to each well. After labeling for 4 hours with [^{3}H] thymidine, CL-1 cells were retrieved in glass filter by cell harvester (Pharmacia LKB). Radioactivity of the glass filters was measured by scintillation counter (beta-plate, Pharmacia LKB). [^{3}H] Thymidine uptake into DNA of the CL-1 cells which were treated similarly to the above, except that they were incubated in the absence of PTH(1-34), was measured as control (indicated as "Medium" in the graph).

2. Results and Discussion

Result is shown below.

Effect of PTH on CL-1



N.S.: Not significant from Medium
(Dunnett multiple comparison)

In the above graph, bars and lines indicate averages ± SD for 3 wells. Statistical analysis using Dunnett multiple comparison revealed no significant difference in [^{3}H]thymidine uptake between the CL-1 cells treated with PTH at various concentrations and the control which were not treated with PTH.

As is clear from the above result, PTH did not inhibit the [^{3}H]thymidine uptake into DNA of the CL-1 cells. In other words, PTH did not stimulate the proliferation of the CL-1 cells of the present invention, which cells were derived from an adult animal.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Dated this 4 th day of June 2001

Hidetomo Kitamura

Hidetomo KITAMURA